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Selective Synthesis of Phosphate Monoesters by Dehydrative Condensation of Phosphoric Acid and Alcohols Promoted by Nucleophilic Bases

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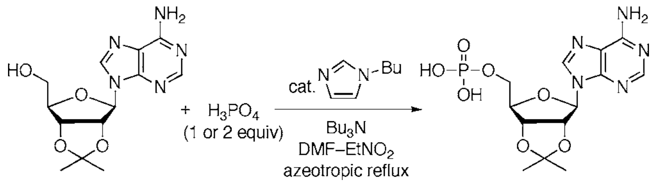
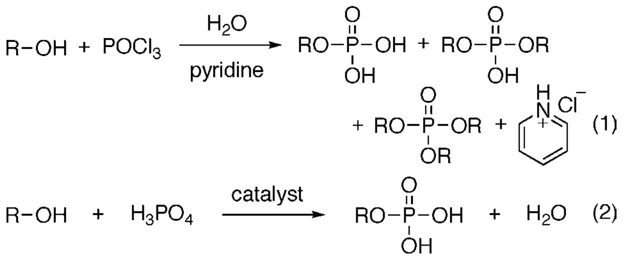
ABSTRACT



Phosphate monoesters are synthesized from a mixture of phosphoric acid (1 or 2 equiv) and alcohols (1 equiv) in the presence of tributylamine. The reaction is promoted by nucleophilic bases such as *N*-alkylimidazole and 4-(*N,N*-dialkylamino)pyridine. 2′,3′-*O*-Isopropylidene ribonucleosides are selectively converted to their 5′-monophosphates without the protection of amino groups in nucleobases.

Phosphate monoesters are some of the most important substances in medicinal chemistry, materials chemistry, and so on. Many methods for preparing phosphate monoesters have been reported.[1] Phosphoryl chloride ($POCl_3$) is one of the most widely used phosphorylating agents for alcohols. The reaction of alcohols with phosphoryl chloride in the presence of water and pyridine provides phosphate monoesters along with pyridine hydrochloride as a byproduct (Scheme 1, eq 1). Since phosphoryl chloride is very reactive, phosphate diesters and triesters are produced as byproducts when alcohols are reacted with an equimolar amount of phosphoryl chloride. Therefore, an excess amount of phosphoryl chloride is required for the selective synthesis of phosphate monoesters. From the perspective of green chemistry, the reaction of equimolar amounts of alcohols and phosphoric acid (H_3PO_4) is more attractive for the synthesis of phosphate monoesters, since the reaction produces only water as a byproduct (Scheme 1, eq 2).

Scheme 1. Synthesis of Phosphate Monoesters



About 40 years ago, Honjo et al. reported the synthesis of 2′,3′-*O*-isopropylidene ribonucleoside 5′-monophosphates from the corresponding ribonucleosides and 5 equivalents of phosphoric acid in the presence of 10 equivalents of tributylamine (Bu_3N) under reflux condition in dimethylformamide (DMF, 153 °C).[2,3] In this pioneering work, an

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(1) For reviews, see: (a) Hayakawa, Y. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Winterfeldt, E., Eds.; Pergamon: Oxford, 1991; Vol. 6, pp 601−630. (b) Slotin, L. A. *Synthesis* **1976**, 737.

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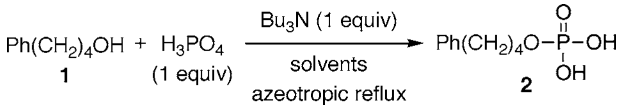
excess amount of phosphoric acid was used and the reaction temperature was very high. Therefore, we investigated a method for synthesizing phosphate monoesters by the direct condensation of equimolar amounts of phosphoric acid and alcohols.

We first investigated the reaction of equimolar amounts of 4-phenyl-1-butanol (**1**) and phosphoric acid in the presence of Bu_3N (1 equiv) in various solvents (Table 1). Reactions were conducted for 6 h under azeotropic reflux with the removal of water (molecular sieves 3A in a Soxhlet thimble). Based on the solubility of phosphoric acid, aprotic polar solvents such as amides, nitroalkanes, and nitriles were thought to be suitable for the reaction.[2] DMF provided phosphate monoester **2** in 70% yield (entry 1). When the reaction was carried out in nitroethane (bp 114 °C), **2** was obtained in 21% yield (entry 2). Nitromethane (bp 101 °C), isobutyronitrile (bp 107 °C), and propionitrile (bp 97 °C) provided **2** in <1% yield (entries 3−5). On the other hand, a nonpolar solvent like *o*-xylene (bp 144 °C) provided **2** in 9% yield because of the low solubility of phosphoric acid in *o*-xylene (entry 6). Our results showed that DMF was the best solvent for the reaction. However, over a prolonged reaction time, DMF decomposed slightly at its boiling point. We therefore investigated the reaction in a mixed-solvent system to lower the reaction temperature and to dehydrate under azeotropic reflux conditions more efficiently. We examined various lower boiling point solvents mixed with DMF and found that a 1:1 (v/v) mixture of DMF−nitroethane was effective, giving **2** in 58% yield (entry 7 versus entry 9). The yield of **2** increased in proportion to the amount of DMF, probably due to the increase in the reaction temperature and the polarity of the solvent (entries 1, 2, 7, and 8). Therefore, we decided to use a 1:1 (v/v) mixture of DMF and nitroethane as the reaction solvent.

Table 1. Optimization of the Solvents in the Reaction of **1** with H_3PO_4[a]

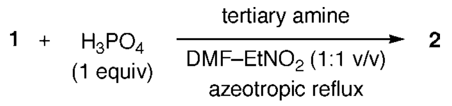


entry	solvent	bp (°C)	conv (%)[b]
1	DMF	153	70
2	$EtNO_2$	114	21
3	$MeNO_2$	101	1
4	*i*-PrCN	107	0
5	EtCN	97	0
6	*o*-xylene	144	9
7	**DMF−$EtNO_2$ (1:1)**		**58**
8	DMF−$EtNO_2$ (1:3)		40
9	DMF−*o*-xylene (1:1)		20

[a] Reactions were carried out with 2 mmol of **1**, H_3PO_4 and Bu_3N in 4 mL of solvents at azeotropic reflux with the removal of water using MS 3A for 6 h. [b] Determined by RP-HPLC and ^1H NMR.

Next, we investigated the reaction of alcohol **1** with phosphoric acid in the presence of various tertiary amines (Table 2). Tertiary amines and phosphoric acid form ammonium phosphates. Lipophilic tertiary amines make phosphoric acid soluble in the solvent. The reaction in the absence of tertiary amine afforded phosphate monoester **2** in 8% yield (entry 1). The yield of **2** increased as the amount of Bu_3N increased (entries 1−3) and reached a plateau at 55−58% when the reaction was conducted with more than 1 equiv of Bu_3N (entries 3 and 4). Triethylamine, which is a less lipophilic tertiary amine than Bu_3N, provided **2** in 37% yield (entry 5), while tertiary amines such as trihexylamine and trioctylamine, which were more lipophilic than Bu_3N, gave results similar to those with Bu_3N (entries 6 and 7). Sterically less hindered *N*,*N*-dimethyloctylamine and more hindered *N*,*N*-diisopropyl-3-pentylamine provided **2** in respective yields of 45 and 44% (entries 9 and 10). Therefore, we decided to use 1 equiv of tributylamine as the tertiary amine.

Table 2. Effects of Tertiary Amines on the Reaction of **1** with H_3PO_4[a]



entry	tertiary amine	equiv	conv (%)[b]
1	none	0	8
2	Bu_3N	0.5	36
3	**Bu_3N**	**1**	**58**
4	Bu_3N	2	55
5	Et_3N	1	37
6	$(n\text{-}C_6H_{13})_3N$	1	62
7	$(n\text{-}C_8H_{17})_3N$	1	60
8	$Me_2NC_8H_{17}$	1	45
9	$(i\text{-}Pr)_2N$(3-pentyl)	1	44

[a] Reactions were carried out with 2 mmol of **1** and 2 mmol of H_3PO_4 in 4 mL of DMF−$EtNO_2$ (1:1) at azeotropic reflux with the removal of water using MS 3A for 6 h. [b] Determined by RP-HPLC and ^1H NMR.

We next examined catalysts for the reaction of alcohol **1** with phosphoric acid (Table 3). An extensive screening of catalysts (10 mol %) showed that some nucleophilic bases promoted the reaction. *N*-Methylimidazole (**3a**), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, **4a**), and 4-pyrrolidinopyridine (**4b**), which are known to be good nucleophilic catalysts, slightly promoted the reaction and gave **2** in respective yields of 63, 60, and 64% (entry 1 versus entries 2, 6, and 7). More lipophilic nucleophiles, including *N*-butylimidazole (**3b**), *N*-phenylimidazole (**3c**), 4-(*N*,*N*-dibutylamino)pyridine (**4c**), and 4-(*N*-hexyl-*N*-methyl)pyridine (**4d**) showed better catalytic activities and gave **2** in respective yields of 67, 68, 67, and 69% because of the good solubility in the reaction mixture (entries 3, 4, 8, and 9).

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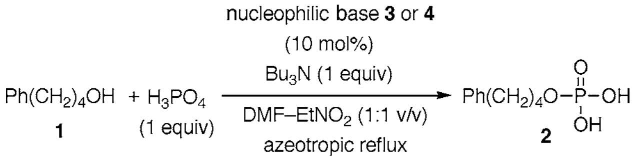
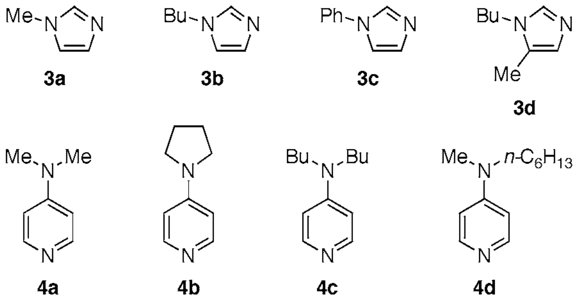
(2) Honjo, M.; Furukawa, Y.; Kobayashi, K. *Chem. Pharm. Bull.* **1966**, *14*, 1061.

(3) Although the 5′-monophosphate of 2′,3′-*O*-isopropylidene uridine (**9**) has been synthesized in 58% yield using the method of ref 2, 2′,3′-*O*-isopropylidene adenosine (**10**), cytidine (**11**), and guanosine (**12**), which are thermally unstable, have been converted to their 5′-monophosphates in respective yields of 38, 39, and 46%.

Table 3. Effects of Nucleophilic Bases[a]





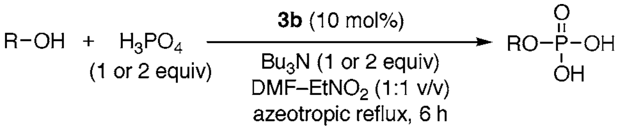
entry	nucleophilic base	conv (%)[b]
1	none	58
2	**3a**	63
3	**3b**	67 (80)[c]
4	**3c**	68
5	**3d**	65
6	**4a**	60
7	**4b**	64
8	**4c**	67
9	**4d**	69

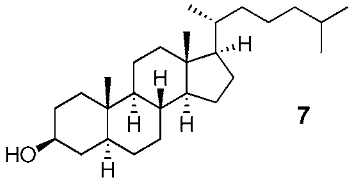
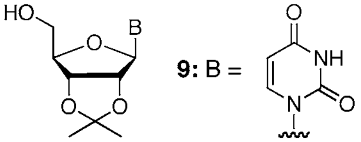
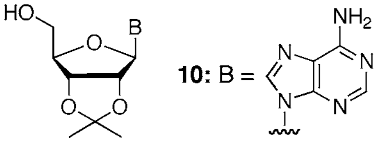
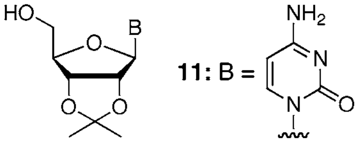
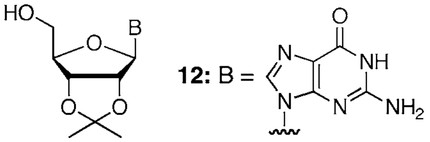
[a] Reactions were carried out with 2 mmol of **1**, H_3PO_4, and Bu_3N in the presence of a catalyst (10 mol %) in 4 mL of $DMF-EtNO_2$ (1:1) for 6 h at azeotropic reflux with the removal of water using MS 3A. [b] Determined by RP-HPLC. [c] The reaction was carried out with 100 mol % of the catalyst.

When 100 mol % of **3b** was used, the chemical yield of **2** was increased to 80% (entry 4). *N*-Butyl-5-methylimidazole (**3d**), which is more nucleophilic than **3b**,[4] had a catalytic activity similar to that of **3b** (entry 5). Therefore, commercially available **3b** was the most suitable catalyst for the reaction although its catalytic activity was still inadequate.

With these optimized conditions in hand, we examined the scope and limitations of the reaction (Table 4).[5] Primary alcohols, such as stearyl alcohol (**5**) and oleyl alcohol (**6**), could be converted to monophosphates in respective yields of 61 and 65% (entries 1 and 2). In the case of secondary alcohols, *β*-cholestanol (**7**) could be converted to monophosphate in good yield (63%, entry 3). When **5**, **6**, and **7** were reacted with 2 equiv of phosphoric acid, their phosphate monoesters were obtained in the respective yields of 84, 87, and 85%. The reaction of a primary amine, such as 4-phenylbutylamine (**8**), did not provide phosphoramidate (entry 4).

Table 4. Synthesis of Phosphate Monoesters[a]



entry	substrate	conv. (%)[b]
1	$CH_3(CH_2)_{17}OH$ **5**	61, 84[c]
2	$CH_3(CH_2)_7CH{=}CH(CH_2)_8OH$ **6**	65, 87[c]
3	 **7**	63, 85[c]
4	$Ph(CH_2)_4NH_2$ **8**	0
5	 **9**: B =	61, 84[c]
6	 **10**: B =	87[c]
7	 **11**: B =	70[c]
8	 **12**: B =	88[c]

[a] Unless otherwise noted, reactions were carried out with a substrate (2 mmol), H_3PO_4 (2 mmol), and Bu_3N (2 mmol) in the presence of **3b** (10 mol %) in 4 mL of $DMF-EtNO_2$ (1:1) for 6 h under azeotropic reflux with the removal of water using MS 3A. [b] Determined by RP-HPLC or ^1H NMR. [c] The reaction was carried out with H_3PO_4 (4 mmol) and Bu_3N (4 mmol).

In the reaction media, amino groups were protonated by phosphoric acid to form ammonium salts, and these ammonium groups were preserved during the reaction. Therefore, we tried to synthesize 5′-monophosphate of ribonucleosides without the protection of amino groups of nucleobases. Since secondary hydroxy groups of ribonucleosides also react with phosphoric acid, 2′,3′-*O*-isopropylidene ribonucleosides were used as substrates. The reactions of 2′,3′-*O*-isopropylidene uridine (**9**), adenosine (**10**), cytidine (**11**), and guanosine (**12**) with 2 equiv of phosphoric acid gave their 5′-monophosphates selectively in respective yields of 84, 87, 70, and 88% (entries 5−8).[3,6] When **9** was reacted with 1 equiv of phosphoric acid, its 5′-monophosphate was obtained in 61% yield (entry 5). No phosphorylation of the amino groups of nucleobases was detected.

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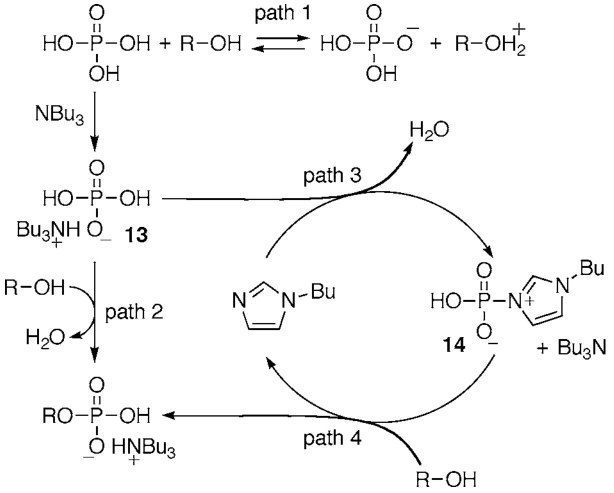
(4) Ishihara, K.; Kosugi, Y.; Akakura, M. *J. Am. Chem. Soc.* **2004**, *126*, 12212.

(5) **General Procedure.** The reaction was carried out in a flask fitted with a pressure-equalized addition funnel (containing a cotton plug and ca. 4 g of molecular sieves 3A and functioning as a Soxhlet extractor) surmounted by a reflux condenser. A solution of an alcohol (2 mmol), phosphoric acid (2 or 4 mmol), tributylamine (2 or 4 mmol), and **3b** (0.2 mmol) in $DMF-EtNO_2$ (1: 1, 4 mL) was heated for 6 h under azeotropic reflux condition with the removal of water. After the reaction mixture was cooled to ambient temperature, tributylamine and **3b** were removed by ion-exchange resin (DOWEX, 50WX2-200). After the solvents were removed, the resulting crude product was analyzed by HPLC (Develosil ODS-HG-5) and/or ^1H NMR.

Next, we discuss the possible mechanism of the reaction (Scheme 2). When the reaction was conducted in the absence of tertiary amine, alcohols were protonated, in part, by phosphoric acid, to decrease the reactivity of alcohol and to give phosphate monoesters in poor yield (path 1). In the presence of an equimolar amount of a tertiary amine such as tributylamine, phosphoric acid and the tertiary amine form ammonium phosphate **13**, which did not protonate alcohols because of its low acidity. Compound **13** still had two hydroxyl groups that could be eliminated and reacted with alcohols to give phosphate monoesters in moderate yields (path 2). Nucleophilic bases, such as **3b**, probably react with **13** to give active species, such as phosphorimidazolidate **14** (path 3). Such an activated species could easily be imagined to react with alcohols to give phosphate monoesters more effectively (path 4).

Scheme 2. Proposed Mechanism of the Phosphorylation



In conclusion, we have developed a method for synthesizing phosphate monoesters by the dehydrative condensation of equimolar amounts of phosphoric acid and alcohols. The reaction was promoted by nucleophilic bases such as **3b** and gave phosphate monoesters in good yield. In this method, phosphoric acid did not react with amino groups. Therefore, the hydroxyl groups of amino alcohols, such as 5′-hydroxyl group of 2′,3′-*O*-isopropylidene ribonucleosides, were phosphorylated selectively. Although several stoichiometric methods for the *O*-selective phosphorylation of *N*-unprotected nucleosides have been reported for the synthesis of nucleoside 5′-monophosphates[2,7] and for the synthesis of nucleoside phosphate diesters,[8,9] the present method is the most atom-efficient and environmentally benign phosphorylation process to synthesize phosphate monoesters selectively.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Pyrophosphoric acid ($H_4P_2O_7$) might also be a good phosphorylating agent for alcohols. However, Honjo et al. reported that the reaction of 2′,3′-*O*-isopropylidene inosine with pyrophosphoric acid gave its phosphate monoester (64%) along with its pyrophosphate monoester (21%) as a byproduct (ref 2).

(7) For the method using pyrophosphoryl chloride ($P_2O_3Cl_4$), see: Imai, K.; Fujii, S.; Takanohashi, K.; Furukawa, Y.; Masuda, T.; Honjo, M. *J. Org. Chem.* **1969**, *34*, 1547.

(8) For the phosphramidite method, see: (a) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395. (b) Sekine, M.; Ohkubo, A.; Seio, K. *J. Org. Chem.* **2003**, *68*, 5478.

(9) For the *H*-phosphonate method, see: Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710.